Markers of Decompression Stress of Mass Stranded/Live Caught and Released vs. Single Stranded Marine Mammals

Michael Moore
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
phone: (508) 289-3228 fax: (508) 457-2089 email: mmoore@whoi.edu

Andreas Fahlman
Department of Life Sciences
Texas A&M– Corpus Christi
6300 Ocean Dr Unit 5892
Corpus Christi, TX 78412
phone: (361) 825 3489 fax: (361) 825 2025 email: andreas.fahlman@tamucc.edu

Award Number: N000141210388

LONG-TERM GOALS

The long-term goal of this study is to develop a biomarker of decompression stress in cetaceans to better understand the link between anthropogenic interactions and barometric stress. We aim to analyze blood samples from captive, wild-caught, and stranded marine mammals in order to compare concentrations of Microparticles (MPs). If confirmed as an indicator of decompression stress, the use of MPs could be applied as a diagnostic tool for stranding events. Development of an effective diagnosis tool has significant implications to the military when the cause of strandings is in question.

OBJECTIVES

Recent necropsy reports have suggested a link between mass stranding of beaked whales and the use of naval mid-frequency sonar (Fernandez et al., 2005). The whales experienced symptoms that were similar to those caused by inert gas bubbles in human divers. These reports have increased the concern that anthropogenic sound, such as that created by military sonar or during seismic exploration, may harm marine animals. It has been suggested that alteration in physiology or diving behavior may increase the risk of decompression sickness (DCS).

Bubble formation is believed to be the crucial event in the etiology of DCS, but the role bubbles play in the disease process remains unclear. As we learn more about DCS, it has become apparent that some of the symptoms are similar to those of other disease states (Kayar et al., 1997; Ward et al., 1990). Recent studies have shown that Microparticles (MPs) correlate with the level of decompression stress in both the mouse (Thom et al., 2011) and human (Vince et al., 2009). MPs are particles between 0.3 to 1 um in size that are shed from various cells. MPs derived from platelets are known to activate leukocytes and cause aggregation, can stimulate pro-inflammatory cytokines, and MPs derived from decompression stress have been shown to activate neutrophils and cause vascular damage (Thom et al.,

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2013		**00-00-2013 to 00-00-2013**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Markers of Decompression Stress of Mass Stranded/Live Caught and Released vs. Single Stranded Marine Mammals	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Woods Hole Oceanographic Institution,Biology Department,Woods Hole,MA,02543**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**5**	

2011). In addition, our recent investigations have confirmed that MPs are present in stranded odontocetes and phocids and that they can be detected by standard assays. Thus, MPs may be suitable biomarkers to assess decompression stress. The study is aimed at verifying a relationship between decompression stress and MPs in sea lions and then transferring this knowledge to assess decompression stress of cetaceans in the field.

APPROACH

This project is separated into three aims:

Aim 1: The relationship between decompression stress and MPs will be calibrated by conducting voluntary dive trials in Steller sea lions housed at the Open Water (OW) Research Laboratory in Vancouver, Canada. By analyzing blood samples before and after a dive bout to depths of 5m and 50m, we aim to verify a correlation exists between MPs and decompression stress (number of dives, duration and depth) in marine mammals. In addition, the metabolic cost (using respirometry), activity (3-axis acceleration data loggers), and environmental variables will be measured during each dive series. B-mode ultrasound will also be used to determine whether bubbles are present before and after the dive bout and correlate the presence of bubbles with the measured decompression stress and MPs.

Aims 2 and 3: We will sample and analyze single and mass stranded dolphins (Aim 2) and live-restrained dolphins (Aim 3) for MPs. We will also collect associated data to determine which stressors correlate with changes in MPs. All available data including MPs, decompression stress (as measured by presence of bubbles defined in Aim 1), environmental variables, standard measures of body condition, and health assessment parameters will be acquired and analyzed using Principal Component Analysis (PCA, Jolliffe IT, 2002, Principal component analysis. Springer, Berlin Heidelberg New York).

Data collected from the calibration study (Aim 1), from stranded animals (Aim 2), from healthy bottlenose dolphins (Aim 3), together with a number of covariates will be analyzed using Principal Component Analysis to assess if elevated MPs are specific to decompression stress in marine mammals.

WORK COMPLETED

Aim 1: In the first year, four adult female Steller sea lions participated in experimental dive bouts to depths of either 5m or 50m at the OW Research Lab. For each experimental dive bout, we collected and analyzed blood samples from each animal pre-dive and post-dive. Preliminary statistical analyses of dive data in the current year support our hypothesis that MPs concentrations are positively related to decompression stress; however stressors such as feeding and exercise may affect MPs levels. Data were collected in the first year to isolate the effect of feeding.

In the second year, surface swim trials were conducted in order to estimate the effects of exercise on MPs levels. By isolating feeding and exercise impacts, we can assess the effects of diving to depth on MPs concentrations. Statistical analyses of surface swim MPs data are currently in progress.

Based on the Steller sea lion experimental dive bout data, an abstract was prepared and submitted to the Society for Marine Mammalogy 2013 Biennial Conference on the Biology of Marine Mammals in

New Zealand. Dr. Fahlman's graduate student Lauren Gonzalez will present this work at the conference in December 2013.

Aim 2: In the second year, blood samples from single and mass stranded marine mammals in Cape Cod were collected and analyzed for MPs concentrations. We plan to collect additional samples from stranded animals in the third year to compare against previous data. Associated morphometric data, environmental variables, and health assessment parameters will be acquired and analyzed using Principal Component Analysis.

Aim 3: In the second year, blood samples from wild-caught dolphins in Sarasota were collected and analyzed for MPs concentrations for Pre-, Mid-, and deck restraint for health assessments. Statistical analyses of these data is ongoing. In the third year, we plan to collect samples from live restrained dolphins in Sarasota, Florida, for additional data analysis. Associated morphometric data, environmental variables, and health assessment parameters will be acquired and analyzed using Principal Component Analysis.

RESULTS

Aim 1: Data were obtained from trained Steller sea lions (4 adult females) wearing time-depth recorders. Sea lions dove to predetermined depths (either 5m or 50m) with blood samples collected pre-dive (0 hrs) and post-dive (3 hrs and 24 hrs). We hypothesized MPs would be positively related to decompression stress. As a proxy for decompression stress, dive depth (dive dose) was integrated for each bout. Statistical analyses indicate that MPs significantly increase after experimental dive bouts ($p < 0.05$). Mean pre-dive (0 hrs) MPs were significantly lower than post-dive (3 hrs and 24 hrs) MPs levels (Fig. 1, Table 1). Since animals were feeding, exercising and diving to depth in these trials, increased MPs levels could be affected by a combination of food, exercise, or decompression stress. In order to isolate the effect of feeding on MPs, we used the same blood sampling protocol and pre-and post-feeding. We found that post-food MPs were 54% greater than MPs without food. Adjusting for this 54% effect of food, there was an average increase in post-dive MPs of 303% at 3 hrs and 458% at 24 hrs (Fig. 2). Although results indicate that food mildly elevates MPs, linear regression analysis demonstrated a significant, positive correlation between dive dose and MPs (Fig. 3).

Preliminary data analyses from the Steller sea lions experimental dive bouts support our hypothesis that MPs concentrations increase as a result of increased decompression stress. Based on surface swim data acquired during the second year, we plan to conduct further statistical analyses in order to isolate the effect of exercise on MPs concentrations.

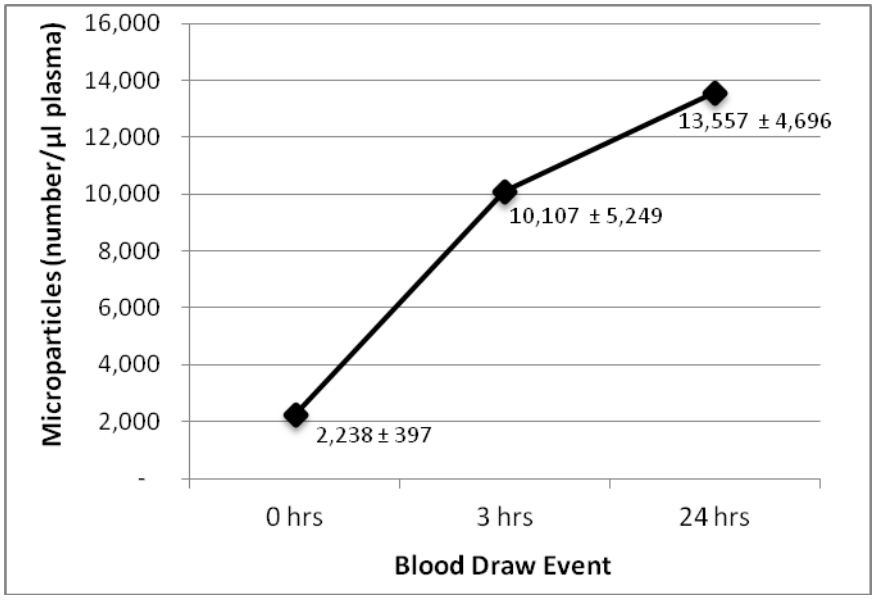


Figure 1. Average MPs for 5m and 50m dive bouts, measured at Pre-Dive (0 hrs), Post-Dive (3 hrs) and Post-Dive (24 hrs)

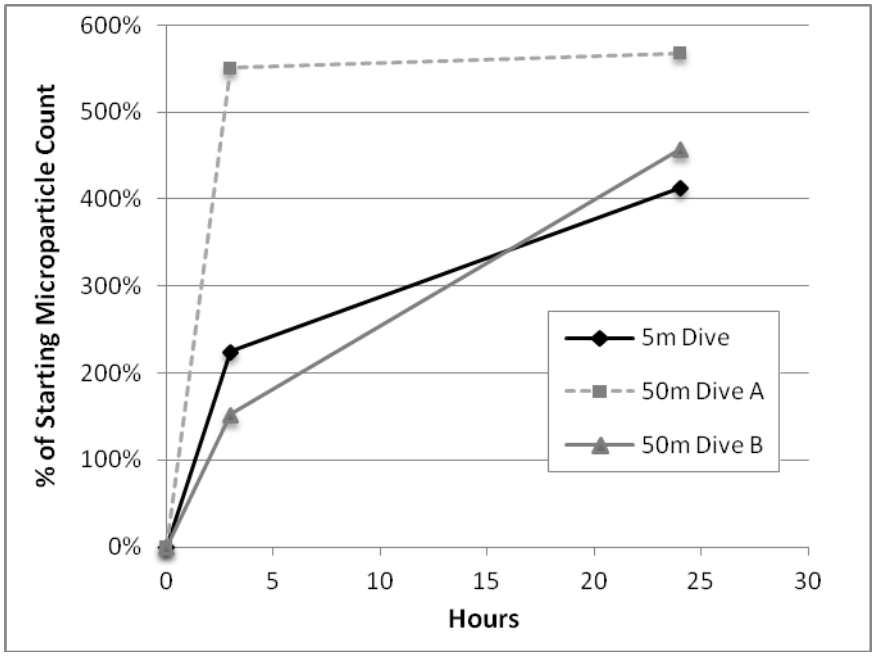
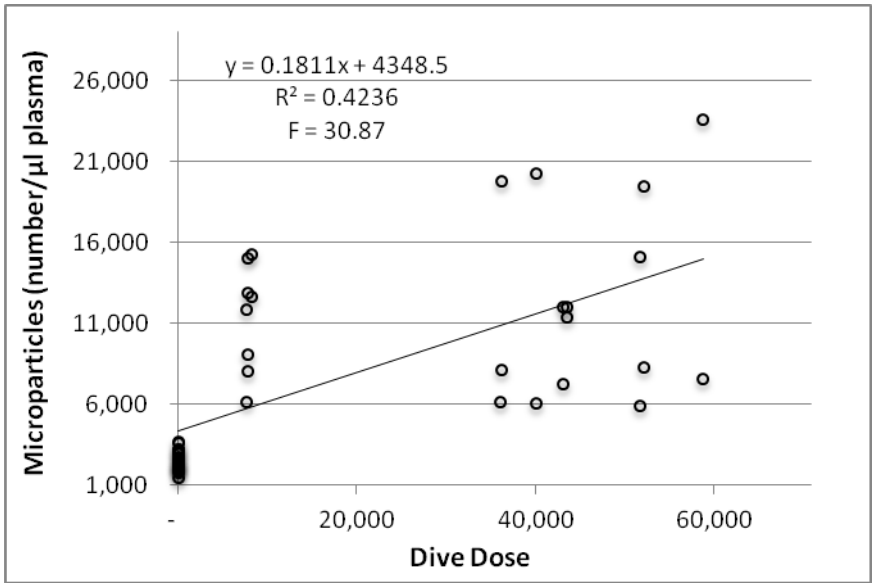


Figure 2: Adjusting for food, percent increase in MPs after Steller sea lions experimental dive bouts at 3 hours and 24 hours

Aim 2: Data analysis pending.

Aim 3: A total of 39 blood samples from 15 wild-caught dolphins in Sarasota, Florida, were analyzed for MPs. The samples were obtained Pre-, Mid-, and Post-procedure. Statistical analysis of data obtained from live-caught dolphins in Sarasota is ongoing; however, preliminary data suggest that relative to Pre-procedure blood samples, MPs may increase in response to out of water stress. There was an average percent increase of 12% and 61%, for Mid-and Post-procedure, respectively. We plan to obtain additional data in the third year.



[*Figure 3. Regression analysis shows positive correlation between MPs and dive dose (estimated by integrating dive depth measurements from time-depth recorder)*]

IMPACT/APPLICATIONS

If confirmed as a biomarker of decompression sickness, MPs can serve as an important diagnostic tool for stranding events. The ability to effectively diagnose decompression sickness in marine mammals will aid in our understanding of anthropogenic sound on marine mammals, which will ultimately improve management practices. This is especially significant in stranding events where the use and role of sonar is in question.

REFERENCES

Fernandez, A., Edwards, J. F., Rodriguez, F., Espinosa De Los Monteros, A., Herraez, P., Castro, P., Jaber, J. R., Martin, V. and Arbelo, M. (2005). Gas and fat embolic syndrome involving a mass stranding of beaked whales (Family *Ziphiidae*) exposed to anthropogenic sonar signals. *Veterinary Pathology* **42**, 446-457.

Kayar, S. R., Parker, E. C. and Harabin, A. L. (1997). Metabolism and thermoregulation in guinea pigs in hyperbaric hydrogen: Effects of pressure. *Journal of Thermal Biology* **22**, 31-41.

Thom, S., Yang, M., Bhopale, V. M., Huang, S. and Milovanova, T. N. (2011). Microparticles initiate decompression induced neutrophil activation and subsequent vascular injuries. *Journal of Applied Physiology* **110**, 340-351.

Vince, R., McNaughton, L., Taylor, L., Midgley, A., Laden, G. and Madden, L. (2009). Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives. *European Journal of Applied Physiology* **105**, 507-513.

Ward, C., McCullough, D., Yee, D., Stanga, D. and Fraser, W. (1990). Complement activation involvement in decompression sickness of rabbits. *Undersea Biomed Res.* **17**, 51-66.